Reprinted from
AMERICAN POTATO JOURNAL, NOVEMBER, 1971, VOL. 48 — No. 11
pp. 428-437

SELECTION POTENTIAL FOR TUBER TOTAL NITROGEN AND TOTAL SOLIDS CONTENT IN A TETRAPLOID BREEDING POPULATION[1]

L. L. SANFORD, T. J. FITZPATRICK, AND W. L. PORTER[2]

ABSTRACT

A 2-year study of 10 crosses involving 20 different tetraploid parents from the USDA potato breeding program showed significant differences in tuber content of total nitrogen (TN, crude protein) and total solids among offspring means and among parents. Offspring TN ranged from 0.20-0.50% in 1968 and from 0.20-040% in 1969 (fresh-weight basis). Heritabilities in the narrow sense ranged from 24-34% (1 and 5 replicates, respectively) for TN and from 24-38% for solids. TN selection was indicated to be potentially as effective as solids selection. The expected gain from selecting 10 of 100 clones in a 5-replicate, 1-year test was calculated as 6.4% of the parental mean for TN and 3.4% for solids.

The potato has been demonstrated to be an excellent food with extremely high-quality protein. The amount of actual protein in cultivated varieties varies from 1.0 to 1.5%. Fitzpatrick, et al. (3) found that a group of breeding lines varied from 1.9 to 3.5% protein when calculated from total nitrogen values, but from 0.9 to 2.2% when protein nitrogen was measured. The high starch-to-protein ratio requires a high caloric intake to furnish the daily requirement of protein (7). If the protein content could be increased, more protein per calorie could be supplied.

The nutritional value of potato protein is as good as, or better than, whole egg, and better than beef, tuna, whole milk, wheat flour, corn, rice, soybean, and kidney bean protein (6). Certain combinations of potato with most of these foods maintained nitrogen balance at much lower dietary nitrogen levels than any food alone. A mixture of 35% whole egg and 65% potato gave the lowest nitrogen intake for maintaining a nitrogen balance ever found by Kofranyi and Jekat (6). These facts and others indicate that potatoes of higher protein content would be an even more valuable food.

The possibility of breeding potatoes for higher protein content has been suggested by Grabner (4), Sengbusch (13), Novak (9), Schupshaw (12), Umaerus (16), and Stegemann (14). Fitzpatrick, et al. (3) found a potential for the development of a potato variety containing increased nitrogenous constituents, both in absolute amounts and in amounts relative to the starch content. However, no work has been found which reports the effectiveness of selection for tuber total nitrogen in a tetraploid breeding population.

---

[1]Cooperative investigations of the Plant Science Research Division, Beltsville, Maryland; and the Eastern Marketing and Nutrition Research Division, Philadelphia, Pennsylvania, of the Agricultural Research Service, U. S. Department of Agriculture. Accepted for publication June 10, 1971.

[2]Research Geneticist, Plant Science Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland; and Research Chemists, Eastern Marketing and Nutrition Research Division, Agricultural Research Service, U. S. Department of Agriculture, Philadelphia, Pennsylvania, respectively.

This paper reports experimental work aimed at understanding the genetic and environmental variation in tuber total nitrogen (TN) and total solids (solids) content for a tetraploid breeding population, and evaluates the potential of TN selection and compares it with that for solids.

Materials and Methods

From the 1966 crosses made in the USDA breeding program at Beltsville, Maryland, 10 were chosen to study the breeding behavior of tuber TN and solids content. The principal criterion for selecting the crosses was avoidance of common parentage. Because the crosses were from a breeding program, they involved selected parents.

First clonal generation tubers were grown in the greenhouse in 1967. Twenty tubers (20 offspring) were randomly picked from each cross, divided into five groups (replications) and planted in a complete, randomized block design at Presque Isle, Maine. Each replicate plot contained four offspring plants with a parent plant at each end. Plant spacings were 2½ feet (0.76 m) and row spacings were 3 feet (0.914 m).

A different offspring sample was randomly picked from each cross in 1969 (second clonal generation tubers), and planted in basically the same design as in 1968, except that three hills per individual offspring and parent were planted instead of one. A replicate-cross group consisted of six three-hill plots (four offspring and two parents) planted randomly in a three-row by two-tier area.

The analysis of main interest is the regression of offspring values on their mid-parent values. The offspring:mid-parent covariance in an autotetraploid, assuming chromosome segregation, equals ½ of the additive genetic variance and 1/6 of the dominance variance (5, p. 408). The mid-parent variance equals ½ of the total genetic variance. Therefore, the regression coefficient equals heritability mostly in the narrow sense. The offspring mean and the mid-parent value in each replicate-cross group were the experimental units in the covariance analysis.

For chemical analysis, therefore, a total of 300 tuber samples were run in each of the two successive growing seasons. All samples were shipped to the Campbell Institute for Agricultural Research, Riverton, New Jersey, where they were stored at 42 F (5.55 C) and 85% relative humidity. About 15 samples at a time were transported to the Eastern Marketing and Nutrition Division in Philadelphia for analysis.

A representative subsample of 500 g (ca. 4 tubers) was taken from each sample by the method reported by Fitzpatrick, et al. (3). A longitudinal wedge (ca. 20% of each tuber) was cut from the stem end to the bud end of each tuber in the subsample. The combined weight of these wedges was about 100 g. The wedge-sample, after accurate weighing, was ground at high speed for 3 min in 300 ml of absolute ethanol in a Waring Blendor.[3] The contents of the blender were emptied into a large beaker equipped with a magnetic stirrer to maintain the homogeneity of the suspension during sampling. Samples for TN analyses were pipetted into

[3]Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U. S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

capped, weighed microkjeldahl flasks and immediately reweighed. Samples (ca. 20 ml) for solids determination were taken by means of a small dipper. These were placed in weighed moisture dishes, capped and reweighed immediately.

The TN analysis was made by using the standard A.D.A.C. microkjeldahl procedure (10) after incorporating modifications in the digestion procedure to prevent excessive foaming (15). The direct solids were determined by heating in a mechanical convection oven at 60 C for one hour, followed by 2 hours at 120 C (3).

Results and Discussion

Fitzpatrick, et al. (3) reported a variation in the ratios of protein nitrogen (PN) to non-protein nitrogen (NPN) of 0.6-2.0 for a group of breeding lines. The NPN fraction contains a fairly large proportion of asparagine and glutamine and, although they are good nitrogen sources for animals, any increase in these compounds will mean a corresponding decrease in the essential amino acids. However, for the purpose of this paper, only TN was studied in order to conserve analytical time. Any promising clones detected by TN analysis would have to be analyzed for asparagine and glutamine contents before a final conclusion could be drawn.

Although both fresh-basis and dry-basis results are available for all TN analyses, only the fresh-basis results were employed in the statistical treatment of the data. Actual data are available from the authors upon request.

The magnitude of TN and solids levels were significantly ($P_{.01}$) different in the two test years for both the parents and offspring. Their interactions with years were not significant for TN but were for solids (Table 1). The differences among parent clones were significant ($P_{.01}$) for TN and solids in both years. Full-sib family means differed significantly ($P_{.01}$) in TN in 1968, but not in 1969; in the combined analyses the differences were significant at $P_{.10}$. For solids, the full-sib families differed significantly ($P_{.01}$) in both years (Tables 1 and 2).

The offspring distributions for TN and solids (Figures 1 and 2) did not deviate significantly from the normal distribution, although the 1968 solids distribution is somewhat skewed toward the high levels. TN values ranged from 0.20-0.50%, which corresponds to a crude protein percentage of 1.25-3.13 (Nx6.25). In 1969, the maximum TN measured was 0.40% or 2.5% crude protein. Approximately 10% of the offspring were higher than this in 1968.

The relative magnitude of genetic variability was measured with genetic coefficients of variation (Table 3). The parent variance components and twice the mid-parent variance component were used as estimates of total genetic variance. The C.V.s utilizing these components range from 10-14% for TN and 5-9% for solids. Twice the mid-parent:offspring covariance component estimates the additive genetic variance plus some dominance. Twice the full-sib family variance component estimates nearly the same quantity except that more intra-locus interaction is present. C.V.s calculated with these components range from 4-6% for TN and 8-11% for solids. Thus, the parents show somewhat more relative total genetic

TABLE 1.—*Estimated variance and covariance components.*

Component	% TN[a]				% Solids[a]			
	1968	1969	Year interaction	Combined years	1968	1969	Year interaction	Combined years
Male Parents	.0015**	.0019**	.00003	.0017**	3.904**	4.469**	.182	4.00**
Female Parents	.0018**	.0021**	.00001	.0020**	1.335**	1.641**	.438**	1.050**
Mid-Parents	.0006**	.0005**	0.0	.0006**	1.570**	1.060**	.125*	1.190**
Full-sib Families	.00023**	.00005	.00004	.00010	2.981**	2.637**	.228*	2.581**
MP:Offspring Covariance	.00018	.00019		.00021	1.750	1.715		1.636

[a] * = $P_{.05}$
** = $P_{.01}$

TABLE 2.—*Two-year average of parent and offspring performance.*

Cross		% TN			% Solids		
Female	Male	Female	Male	Offspring mean	Female	Male	Offspring mean
B4808-8 x B3692-4		.354	.325	.324	21.28	20.18	19.87
B725-61 x B3819-17		.328	.247	.319	19.63	21.80	19.17
Norgold Russet x B3478-45		.350	.322	.318	21.80	21.83	21.50
Houma x B5141-6		.307	.339	.336	21.56	26.64	24.42
Kennebec x Katahdin		.321	.343	.336	22.14	22.11	20.48
Ona x B5236-8		.334	.320	.323	21.08	20.02	19.79
B4557-2 x Merrimack		.231	.332	.307	21.03	22.44	20.44
Wauseon x Seminole		.334	.387	.345	20.68	24.50	23.13
B5052-14 x B5042-2		.284	.381	.313	19.09	24.48	19.87
B5281-1 x B5011-17		.402	.280	.343	18.80	21.91	20.04
Mean		.324	.328	.326	20.71	22.59	20.87
S.E. of Mean		.009	.008	.016	.53	.46	.94

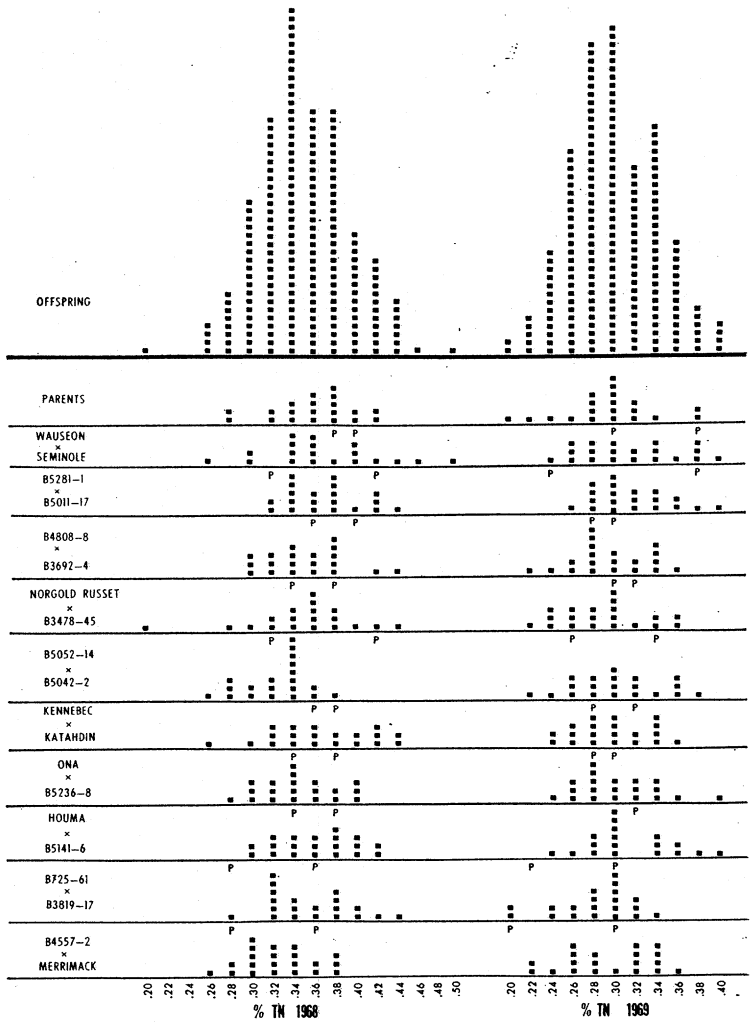
potential for TN than for solids content, but less relative breeding value potential (additive genetic variance).

Although the parents were not chosen by the authors because of their solids content, the fact that they were used as parents in the breeding program indicates that they had been subjected to such selection. Clones with low solids content are not likely to survive to the point of parental use. Thus, such a sample of parents would tend to be similar in solids content.

Heritabilities:

The mid-parent:offspring covariance components for TN and solids were quite consistent over years (Table 1). The regression coefficients for TN and solids are significant ($P_{.05}$) in the pooled analysis, but not in

Figure 1. DISTRIBUTION OF OFFSPRING AND PARENTS FOR % TN [a]

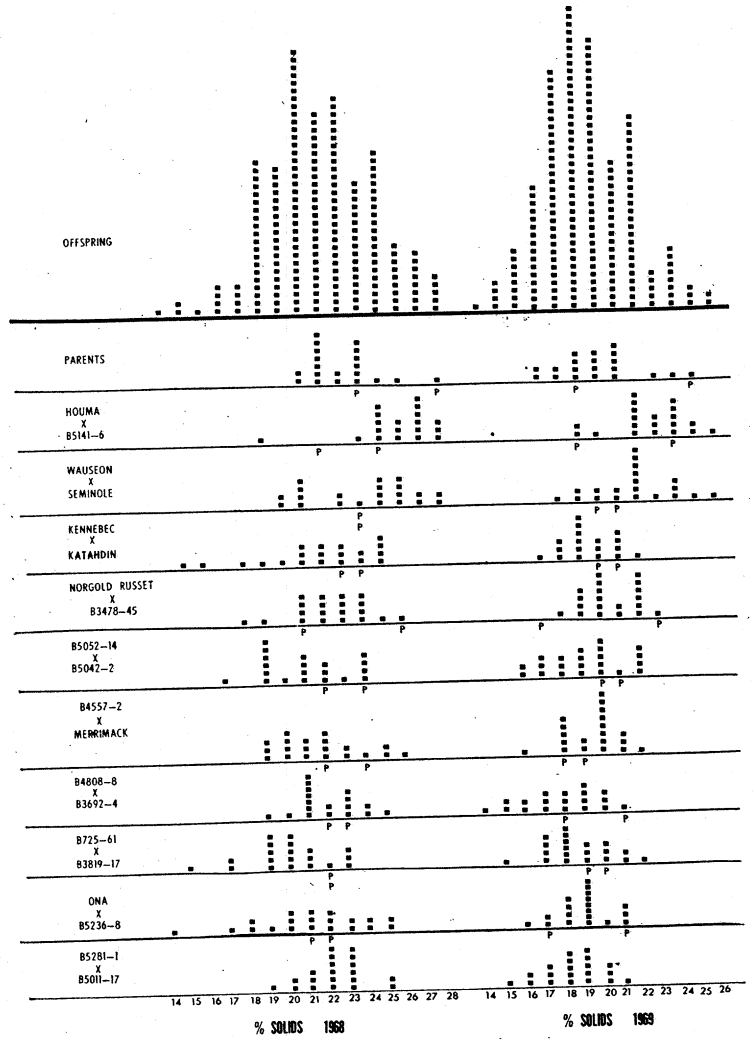


a] Each dot represents one offspring or parent; P marks a parent mean.

individual years. The deviations from regression are significant ($P_{.05}$) only for solids.

As stated previously, heritability estimated from the regression of offspring on mid-parent is mostly in the narrow sense and equals the regression coefficient. On a 1-year base, this heritability is smaller for TN than for solids (Table 4). Most of the estimates for solids exceed 1. A possible reason for this is the selected nature of the parents for solids. The mid-parent variance is consistently lower than the full-sib family variance (Table 1), and the offspring means are generally lower than their mid-

FIGURE 2. DISTRIBUTION OF OFFSPRING AND PARENTS FOR % SOLIDS [a]



a) Each dot represents one offspring or parent; P marks a parent mean.

parent values. Fig. 2 shows the considerable number of offspring that fall below the parent averages. Other workers (1, 8) also have reported this type of results for solids.

According to Falconer (2, p. 170), selection of parents should not affect the regression coefficient because the covariance is changed to the same extent as the parent variance. However, Kempthorne (5, p. 329) states that this is true only if the regression line is linear throughout; i.e. there are no dominance deviations. Non-linearity seems to be present in

TABLE 3.—*Genetic coefficients of variation.*[1]

Measurement	(Vg) ½/mean		(2Vmp) ½/mean	(2Voff) ½/mean	(2Cov.) ½/mean	
	Male mean	Female mean	Mid-Parent mean	Offspring mean	Offspring mean	Mid-Parent mean
	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
% TN	12	14	10	4	6	6
% Solids	9	5	7	11	9	8

[1] V_g = parent variance component
V_{mp} = mid-parent variance component
Cov. = covariance component of mid-parent:offspring
V_{off} = full-sib family variance component

TABLE 4.—*Heritability estimates on a 1-year basis.*

Type of estimate	% TN		% Solids	
	1 Replicate	5 Replicates	1 Replicate	5 Replciates
	Pct.	Pct.	Pct.	Pct.
Broad Sense				
Male Parent	77	93	74	90
Female Parents	71	92	50	65
Mid-Parents	63	89	64	84
Narrow Sense				
Off.:MP Regression	24	34	88 (24)[2]	>1 (38)[2]
Full-sib Family[1] Variance Component	10	15	>1 (28)[2]	>1 (55)[2]

[1] The denomenator (phenotypic variance) of this estimate includes the maximum variance component estimates of error and interaction for the male and female parents, plus twice the mid-parent variance component. The numerator is twice the full-sib family variance component.
[2] Houma x B5141-6 and Wauseon x Seminole crosses excluded.

these solids data and is primarily due to two crosses. The offspring from Houma x B5141-6 and Wauseon x Seminole averaged considerably higher in solids than did the offspring from the other crosses, and they were the only ones with larger means than their mid-parent values (Table 2 and Fig. 2). The solids heritability calculated excluding these two crosses is considerably reduced and is nearly equal to the TN heritability (Table 4). A point of interest here is that the male parents in these crosses, B5141-6 and Seminole, are full-sibs and have *S. chacoense* Bitt. in their pedigree.

The selected nature of the parent sample for solids does not seem to have affected TN response to any appreciable extent as indicated by: (i) offspring means and their mid-parent values are about equal; and (ii) offspring and parent genetic variance are about equal. The offspring genetic variance was estimated by summing the between full-sib family

TABLE 5.—*Expected genetic gain in the next generation expressed as the percentage of the parent mean.*[1]

	Phenotypic standard deviation	Heritability (narrow sense)	Genetic gain
			Pct.
% Solids[2]			
1 Replicate	1.3982	.237	2.7
5 Replicates	1.1103	.376	3.4
% TN			
1 Replicate	.04207	.241	5.4
5 Replicate	.03515	.345	6.4

[1]Ten clones selected from 100 (standardized selection differenial = 1.73) in 1-year test.
[2]Houma x B5141-6 and Wauseon x Semniole crosses excluded.

and within full-sib family variance components and this equals 0.00172 (common environmental variance within plots is included in this value). The parent genetic variance was estimated by doubling the mid-parent variance component and this equals 0.00110. In contrast, the offspring and parent genetic variance estimates for solids are widely different, 2.38 and 6.23, respectively.

The full-sib family variance component can also be used to estimate heritability (narrow sense). For TN, this estimate is somewhat smaller than the regression estimate even though the full-sib family component theoretically contains more intra-locus interaction variance (Table 4). Again, solids heritability exceeds 1.0 when all crosses are included, but ranges from 28-55% when the two high crosses are excluded.

Heritability in the broad sense, calculated from parent and mid-parent variances (Table 4), is roughly equal for TN and solids, and is about three times larger than the narrow sense values.

Expected selection progress:

Plaisted and Peterson (11) completed two cycles of phenotypic recurrent selection for high specific gravity and gained .004 units. They tested at two locations in 3 years. They measured their gain between the parent means of cycles 1 and 2. This response, therefore, is the result of total genetic variance. The gain from the unselected population mean of cycle 1 to that of cycle 2, which was not measured, probably would be smaller because the total genetic variance would not be expressed. Their gain, expressed as a percentage of the cycle 1 parent mean, equals 4.1 (percent solids scale).

As an example of what genetic progress the present results indicate might be made, Table 5 presents the expected gain in the generation after mating 10 clones selected from 100 clones tested in one year. The values for solids are roughly comparable with the 4.1% gained by Plaisted and Peterson. The comparison is only approximate because their selection pressure differed in each year, and because not all genotypes were tested in all environments. Also, of course, they were selecting on the basis of total gen-

etic variance. They concluded that specific gravity could be effectively increased by phenotypic recurrent selection.

The expected genetic response calculated for TN (Table 5) suggests that its selection may be as effective as for solids in percentage change from the parental mean. The expected gain is actually somewhat greater for TN, but the nature of the sample population with respect to solids causes the authors to view this with some reservation. Genetic variance for solids is rather low in this sample, particularly after excluding the two high crosses to obtain a valid heritability estimate. The additive proportion of the genetic variance (Cov. component/M.P. component) is greater for solids (63%) than for TN (39%), but the large genotype x year interaction for solids (small for TN) lowers its expected genetic response.

Because TN is included in total solids, their measurements should vary together to some extent, but being only a small portion of solids, TN changes only slightly compared with changes in solids associated with genotype or environment. Simply selecting for increased solids would increase TN very little (3). This kind of selection would primarily increase starch content and thus increase the ratio of starch to protein. A low ratio would be preferable to a high ratio because more of the tuber's total energy would be represented by protein (3). If TN were selected on a dry-weight basis, low ratios would result. However, there would be a strong tendency to select for lower solids, because the starch content range is much larger than the TN range; and consequently the lower ratios would be mainly the result of solids content. Therefore, TN probably should be selected for on a fresh-weight basis, or on a dry-weight basis within a narrow solids range.

These results indicate that, within the tested population, there exists genetic variability for TN content of sufficient magnitude to allow improvement by selection. The effectiveness of such selection should be similar, proportionately, to that for solids content, which from breeding experience and experiments has been shown to be moderately successful.

Literature Cited

1. Cunningham, C. E. and F. J. Stevenson. 1963. Inheritance of factors affecting potato chip color and their association with specific gravity. Amer. Potato J. 40: 253-265.
2. Falconer, D. S. 1960. Introduction to quantitative genetics. The Ronald Press Co., New York. 365 p.
3. Fitzpatrick, T. J., R. V. Akeley, J. W. White, Jr., and W. L. Porter. 1969. Protein, nonprotein, and total nitrogen in seedlings of potatoes. Amer. Potato J. 46: 273-286.
4. Grabner, E. 1907. Experiments on the hereditary transmissions of the potato. Z. Landw. Vers. Wes. Oesterr 10: 607-647. CA 2: 297.
5. Kempthorne, O. 1957. An introduction to genetic statistics. John Wiley and Sons, Inc., New York. 545 p.
6. Kofranyi, E. and F. Jekat. 1965. Zur Bestimmung der biologischen Wertigkeit von Nahrungsproteinen, XII. Die Mischung von Ei mit Reis, Mais, Soja, Algen. Hoppe Seyler's Z. Physiol. Chem. 348, 84-88.
7. Kon, S. K. and A. Klein. 1928. XXXV. The value of the whole potato in human nutrition. Biochem. J. 22: 258-260.
8. Maris, B. 1969. Studies on the maturity, yield, underwater weight, and some other characteristics of potato progenies. Euphytica 18: 297-320.
9. Novak, F. 1960. The protein content of potatoes. Sboraik Ceskoslov. Akad. Zemedel. ved. Rostlinna Vyroba. 6: 1247-1260. CA 55: 7558d

10. Official Mehods of Analysis of the A.O.A.C. 1965. Association of Official Agricultural Chemists. 10th Ed. 957 p.
11. Plaisted, R. L. and L. C. Peterson. 1963. Two cycles of phenotypic recurrent selection for high specific gravity. Amer. Potato J. 40: 396-402.
12. Schupshaw, W. 1970. Control of plant proteins: The influence of genetics and ecology of food plants. Pages 245-265 *in* "Proteins as human foods." AVI, Westport, Conn. 525 p.
13. Sengbusch, R. V. 1941. Die Bedeutung der Eiweissleistung der Kartoffel. Forschungsdienst 12: 517-532.
14. Stegemann, H., V. Loesschcke, and B. Lerch. 1967. Biochemische Möglichkeiten zur Beurteilung der Pflanze. Gesunde Pflanzen 19: 192-196.
15. Talley, E. A., T. J. Fitzpatrick, W. L. Porter, and H. J. Murphy. 1961. Chemical composition of potatoes. 1. Preliminary studies on the relationships between specific gravity and the nitrogenous constituents. J. Food Sci. 26: 351-355.
16. Umaerus, M. 1969. Potatisens proteininnehall. Sver. Utsaedesfoeren Tidskr. 79 (3-4): 256--266.